



Dehydrated *Maydis stigma* Enhances Nutritional and Antioxidative Capacities of Butter Cookies

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ABSTRACT

Background: Maydis stigma is generally disposed after taking the cornettes as vegetable. **Objective:** In the present study, Maydis stigma powder (MSP) was partially replaced with wheat flour at the levels of 5, 10 and 15% in cookies preparation. Nutritional compositions were measured using standard methods and instrumental texture properties were also determined. Polyphenol compounds of cookies was determined using High Performance Liquid Chromatography technique while antioxidative activities were performed using FRAP and DPPH methods. **Results:** The incorporation of MSP in cookies increase the amount of protein, dietary fiber and ash content compared to cookie prepared from wheat flour (control). The present study also demonstrated that considerable higher polyphenol content (60.4% to 86.8%) and antioxidative improvement has attained by incorporation of MSP in cookie formulations. The MSP-based cookies have stronger free radical scavenging capacity (24.45 % to 62.73%) and ferric reducing capacity (16.94 to 342.00 $\mu\text{mol TE/g}$) with higher gallic acid and ferulic acid compound compared to all-wheat based cookies. **Conclusion:** Cookies with high antioxidative capacities can be prepared by replacing wheat flour with MSP up to 10%. Thus, MSP is suggested as potential nutritional and natural antioxidant sources for processed food products.

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INTRODUCTION

Bread and biscuit are categorized amongst 10 most popular food products consumed daily in Malaysia [1]. Biscuit for instance is a favorable bakery product and usually served at different occasions. Moreover, biscuit is also considered as a good source of energy that provides better supplementation vehicle for nutritional improvement based on three major ingredients such as flour, sugar and fat [2]. Unfortunately, bakery products available on shelf are high in fat and low in dietary fibre thus limit their consumption in the diet [3]. Therefore, the incorporation of dietary fibre in bakery products has become of great interest due to the potential health benefits conferred by both soluble and insoluble fibres. Increased intake of dietary fibre in diet has been known to reduce the risk of colon cancer, diabetes, obesity and cardiovascular diseases [4]. Hence, the incorporation of dietary fibre in processed food could help to increase the dietary fibre intake. In this regard, it is essential to increase utilization of dietary fibre of various sources in bakery products such as biscuit. For instant, biscuit formulated with orange and mango peel had improved the dietary fibre content instead of good consumers' acceptance [5, 6]. Biscuit enriched with polyphenols containing bioactive compounds may increase the nutraceutical properties of the product [6]. Moreover, other nutritional composition contained in plant sources has improved desirability from consumers [3].

Cornsilk is a long, yellowish stigma of a corn fruit. Cornsilk is normally discarded while its nutritious young corn ear is taken as vegetable. Averagely, a young corn ear produces the silks which comprise 25 % of its fresh weight. Thus, it is estimated that a young corn ear would probably produce nearly 50,000 tonnes of silks annually. In old centuries, cornsilk has been used as a traditional medicine to cure several illnesses associated with the urinary system, prostate problems, bed-wetting, carpal tunnel syndrome, edema and obesity [7]. In regards to its nutritional aspect, cornsilk contains protein, vitamins, carbohydrate, minerals, volatile oils, steroids (sitosterol and stigmaterol), alkaloids, saponins, and tannins [8]. This plant byproduct also contains polyphenol compounds such as flavonoids and showed a strong antioxidant activity [7, 8, 9]. To date, there is no such effort

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in utilizing young corn ear silk or cornsilk in bakery products such as biscuit. Therefore, the objectives of this study are to determine the influence of cornsilk on nutritional composition, texture and colour properties and antioxidative capacity of butter biscuit.

MATERIALS AND METHODS

Plant material:

Cornsilk (1 kg) were chopped and spread thin on trays before dried in an oven (Memmert, Germany) at 55° C for 2 days. Dried silks were ground into powder form (CSP) (Waring Commercial, USA) and sieved through a 500 mm sieve.

Formulations of biscuits:

The ingredients used for making biscuit were flour (160g), butter (64g), an egg, sugar (36g) and sodium bicarbonate (2g). Butter, sugar and egg were creamed in a mixing bowl using an electric hand mixer (National, Japan). Dry ingredients such as flour, cornsilk powder and baking powder were sieved and mixed into the batter. For the production of CSP supplemented biscuit, blends of flour and CSP at a proportion of 5%, 10% and 15% were prepared. The dough was blended sheeted to approximately 5 mm thickness on a flat surface. All biscuits were baked in an oven at 170 °C for 15 minutes and kept in a sealed plastic pouch.

Proximate analyses:

The biscuits were analyzed for moisture, crude protein, fat and ash using standard methods [10]. In protein determination, a nitrogen-to-protein conversion factor of 6.25 was used. TDF content was determined by enzyme gravimetric method [10]. Carbohydrate content was calculated by difference.

Physical, texture and colour characteristics of biscuit:

Diameter (W) of biscuits was measured and average values of these biscuits were reported in millimeter. Thickness (T) of biscuits was measured by stacking ten biscuits on top of one another. The biscuit spread ratio was calculated by dividing diameter (W) by thickness (T). Texture was expressed as breaking strength (kg force) and was measured using a Texture Analyzer (TA-HDi, Stable Microsystems, UK) using 5 kg load cell. In colour analysis, the L, a, b values were determined using a spectrophotometer (Minolta, CM 5084, Japan).

Polyphenols content and antioxidative activity:

Crushed biscuit (20 g) was defatted with hexane. The biscuit was homogenized twice with MeOH in the ratio of 1:10 (w/v) for 10 min at 7000 rpm. The extract was pooled and centrifuged at 10000 rpm for 5 min before supernatant was made to 10 ml with solvent. Polyphenol content was determined using a Folin-Ciocalteu method [11]. One ml of the extract was added with 1 ml of Folin-Ciocalteu reagent (1:1) (Merck, USA). Sodium bicarbonate (20 %, w/v) (1.5 ml) was added and the flask was made to 10 ml with distilled water. After 2 hours, the absorbance of sample was recorded at wavelength 765 nm by using UV-VIS spectrophotometer (Cary, Varian, USA). For the standard reference, gallic acid (Sigma, USA) was prepared in five concentrations ranging from 6.25 to 100 µg/ml. The total polyphenol content was calculated from the linear equation of gallic acid calibration curve and expressed as mg gallic acid equivalent (GAE)/g extract.

Five g biscuit was extracted with MeOH and subjected for DPPH free radical scavenging assay. One milliliter of extract or BHT was added with 4 ml DPPH solution (1 Mm). The sample was allowed to react in the dark for 30 minutes at room temperature and absorbances were recorded at 520 nm by using UV-Vis spectrophotometer (Cary, Varian Inc., USA). The radical scavenging capacity of biscuit was expressed as percentage of inhibition and calculated as follow:

$$\text{Inhibition (\%)} = (\text{Abs (neg control)} - \text{Abs sample}) / \text{Abs (neg control)} \times 100$$

where Abs (neg control) = absorbance of the negative control

Abs sample = absorbance of test sample.

For FRAP capacity, the methods were carried out according to [12]. One milliliter of the extract (Section 2.5.3) was transferred into a 15 ml centrifuge tube and added with 2.5 ml of phosphate buffer (0.2 M, pH 6.6). An aliquot of 2.5 ml of K₃Fe(CN)₆ (1%, w/v) was added to the tube and sample was heated at 50° C for 20 minutes. After which, 10 % TCA (w/v) (2.5 ml) was added, vortexed and centrifuged at 3000 rpm for 10 minutes. An aliquot of 2.5 ml was taken out and transferred into another tube which was filled with 2.5 ml of distilled water. FeCl₃ (0.1 %, w/v) (0.5 ml) was added and the tube was left to react for 10 minutes at room temperature. Absorbance was measured at 700 nm by using UV/VIS spectrophotometer and FRAP capacity was reported as Trolox equivalent antioxidant capacity (mg TE/100g product).

Biscuit sample was extracted according to [13] with some modifications. Five grams of defatted biscuit sample was extracted twice with 25 ml of MeOH (80 %, v/v) instead of ethanol (80 %, v/v). The sample was homogenized for 10 min at 7 000 rpm (UltraTurrax, Ika, Germany) and centrifuged at 10 000 rpm for 5 minutes.

The extract was vacuumed evaporated at 40° C and further dried under N₂. Biscuit extract was dissolved in MeOH/mobile phase (80:20, v/v) and the chromatographic condition was set as described in Section 2.6.1.

HPLC instrument (Gilson, Inc, USA) was equipped with UV-VIS detector (Gilson 151, USA) and a pump (Gilson 307, 10s, USA). The mobile phase contained 15% acetonitrile (Merck) and 85% of sodium phosphate solution (0.01 M), acidified to pH 3.2 with 0.1 M ortho-phosphoric acid (Merck, USA). The chromatography was performed on a C18 column (150 mm in length x 4.6 mm diameter x 5 µm pore size) (Lichrospher, USA) equipped with a guard column (Lichrospher, USA). The extract (20 µl) was injected and the chromatography was set at flow rate 0.75 ml/min at wavelength 280 nm in triplicate. The phenolic compounds present were expressed as µg/g sample.

RESULTS AND DISCUSSION

Proximate composition:

The biscuit containing CSP showed higher moisture, protein, ash and TDF and lower carbohydrate content compared to the control biscuit. The biscuit incorporated with 5% to 15% of CSP contained significantly higher moisture (2.08% to 4.05%), protein (8.74% to 9.81%) and ash (0.81% to 1.07%) compared to the control biscuit (Table 1). Fat content among control and all biscuits containing CSP was not significantly different whereas the carbohydrate content showed a significant decreasing trend from 64.06% to 58.24% as higher amount of CSP was incorporated. The highest carbohydrate content was shown by the control biscuit (66.76%). Biscuit incorporated with 5% to 15% of CSP showed significantly higher content of TDF (2.55% to 5.30%) than the control biscuit (1.14%).

Table 1: Proximate composition of biscuits incorporated CSP.

Nutritional components (%)	CSP level (%)			
	0 (Control)	5	10	15
Moisture	1.94±0.04 ^d	2.08±0.02 ^c	3.11±0.08 ^b	4.05±0.02 ^a
Fat	21.26±0.41 ^a	21.75±0.32 ^a	21.93±0.23 ^a	22.16±0.26 ^a
Protein	8.03±0.21 ^c	8.74±0.22 ^b	8.98±0.11 ^b	9.81±0.12 ^a
Ash	0.68±0.04 ^c	0.81±0.04 ^b	0.88±0.03 ^b	1.07±0.02 ^a
¹ Carbohydrate	66.76±0.46 ^a	64.06±0.34 ^b	60.87±0.52 ^c	58.24±0.44 ^d
TDF	1.14±0.10 ^c	2.55±0.22 ^b	4.25±0.28 ^a	5.30±0.38 ^a

^{a-d}Superscript of different letter within the same row was is different significantly at P≤0.05.

¹By difference

Moisture, fat, protein, ash and TDF content of biscuit incorporated with CSP have increased with the increased level of CSP used in formulations. It has been reported that the increase of macronutrient in finished product could be due to the complementation of wheat flour with other replacements containing higher amount of that nutrients [14]. In this study, CSP contained higher moisture (4.15%), fat (1.27%), protein (12.96%), ash (5.28%) and TDF (48.50%) content than that of wheat flour. Wheat flour was reported to contain 1.34g/kg moisture, 0.14g/kg fat, 1.07g/kg protein, 0.14g/kg ash and 0.84g/kg TDF [15]. Therefore, the increase of moisture, protein and ash content in CSP biscuits could be attributable to the higher content of these nutrients in CSP.

Moisture content of biscuit incorporated with CSP in this study was lower than other biscuits substituted with plant byproducts or other edible plant sources. In a form of dough, the moisture was contributed by amount of water added and also from naturally occurring water presences in the ingredients which could range from 11-30% [16]. The total moisture content of biscuit should not exceeding 5 % of moisture content was the most appropriate level for biscuit in order to maintain its shelf life. As the thermal processing is completed, the moisture content could reduce to 1% to 5% in the final products [16].

The moisture content of CSP biscuit had increased with the inclusion of higher level of CSP. The increase of moisture content in these biscuits could be attributable to the stronger binding capacity of the by-product at higher level of incorporation [17]. It was reported that the differences in water absorption was attributed to the greater number of hydroxyl group presence in the fibre structure which eventually allows more water interaction through hydrogen bonding [18]. Moisture content of CSP biscuits studied here was comparable with the biscuit containing 10% to 20% of finger millet seed coat (3.5 % to 4.2 %) [19], 2.5% to 7.5% of *Tinospora* leave powder or guduchi (4.18% to 4.53%) [20] and 12% flaxseed flour [21], but lower than the biscuits incorporated with dehulled pigeon pea (PPDF) (4.08% to 4.81%) and pigeon pea byproduct flour (PPBF) (4.02% to 4.83%) at 5 % to 25 % of flour substitution [22]. However, the CSP biscuits showed higher moisture content than the biscuit incorporated 5% amaranth leaves powder (1.87%) and processed pearl millet (*Pennisetum glaucum* L) (2.57 % to 2.67 %) [23].

Fat imparts desirable eating qualities that also contribute to texture and flavour of the products [24]. It also functions as lubricant which contributed to elasticity of the biscuit dough during processing and prevents excessive development of gluten proteins [24]. The fat content obtained in the present study was not altered regardless of amount of CSP in the biscuits (0% to 15% of CSP). This could be due to the same amount of fat

being used in all formulations and low fat composition of CSP itself (1.27%). The fat content of CSP-based biscuit was in agreement with the biscuit substituted with 5% to 25% of Indian brown mustard (*Brassica juncea*) [17], sorghum and soy biscuit [25]. However, the content was slightly higher than the biscuit containing pigeon pea biscuits (19.19% to 19.75%) [22].

Protein content of biscuit was increased by substitution of wheat flour with CSP. The increased of protein content could be attributable to the complementation of wheat flour with other replacements that contains higher amounts of protein [14]. Protein content of CSP biscuits was higher than the pigeon pea biscuits (7.28 % to 8.64 %) [22], oyster mushroom biscuit (6.66% to 6.94%) [26] and the orange peel biscuit (5.53 % to 7.48 %) [27]. However, the biscuit replaced partially with millet and pigeon pea flour contained higher protein content (12.1% to 14.4%) than CSP-based biscuit because these two types of flour contained considerably high protein composition that was 11.3% and 22.5% respectively [28]. Decrease in protein content usually corresponds to the increase level of carbohydrate content [27]. The carbohydrate content in CSP-based biscuit was found lower than pigeon pea biscuit (66.37%) at the same level of flour substitution (5 %). On the other nutrient, CSP biscuit had comparable amount of ash content with those pigeon pea biscuits (0.78 % to 1.23 %).

Physical and textural properties:

The hardness of biscuits was influenced by different level of CSP. The hardness value of control biscuit (1600±189 N) was not comparatively different with biscuit containing the lowest level of CSP (5%) (1649±134 N). However, an increasing trend in hardness value was shown by the biscuit containing 10 % (2069±161 N) and 15% CSP (2464±152 N). The diameter and thickness of the control and biscuit incorporated with 5% and 10% CSP was not significantly different (Table 2). The spread ratio of control and CSP biscuits was not significantly different when added with 5% and 10% of CSP. Meanwhile, the diameter (W) was decreased but the thickness increased significantly in 15 % CSP biscuit.

Table 2: Texture, physical properties and water activity of CSP biscuit.

Parameters	CSP level (%)			
	0	5	10	15
Diameter (W, mm)	28.03±0.18 ^a	28.35±0.48 ^a	28.06±0.25 ^a	27.13±0.35 ^b
Thickness (T, mm)	5.06±0.25 ^b	5.01±0.18 ^b	5.00±0.26 ^b	5.16±0.37 ^a
Spread ratio (W/T)	5.55±0.24 ^a	5.65±0.26 ^a	5.63±0.35 ^a	5.28±0.31 ^b
Hardness (N)	1600±189 ^c	1649±134 ^c	2069±161 ^b	2464±152 ^a
Water activity	0.31±0.01 ^d	0.38±0.02 ^c	0.46±0.01 ^b	0.54±0.02 ^a

^{a-c} Superscript with different letter within the same row was significantly different at $p < 0.05$.

In the present study, incorporation of CSP at 15 % level had significantly decreased biscuit spread ratio. In addition, incorporation of 5 to 10 % CSP did not significantly affect the spread ratio. The reduction could be attributable to competition for water between wheat flour and CSP in order to reach dough consistency [22]. In addition, the chemical composition in food composite may have compete for the available water during dough mixing which resulted in decreased of spread ratio [29] as could be seen in higher CSP substitution. Similar trends of decreasing spread ratio in biscuit were also reported in biscuit incorporated with palm (*Archontophoenix alexandrae*) flour [30]. The biscuits which were substituted with 10 % to 25 % of palm flour had significantly decreased in height (0.60 to 0.55), diameter (3.17 to 2.84) and spread ratio (5.28 to 5.21) compared to the control biscuit as higher level of palm flour was incorporated [30]. Cookie spread has decreased significantly in biscuits substituted with combination of rice bran and soy sources such as defatted soyflour (7.9 to 8.0), non-defatted soyflour (8.3 to 8.8), soy concentrate (6.8 to 7.2) and soy isolate (7.4 to 7.7) compared to the control biscuit (9.8) [31]. The biscuits prepared with wheat and rice brans showed a decrease in spread ratio from 8.30 to 7.73 and 8.02 to 7.52 respectively. In contrast, [32] reported that biscuit prepared with sunflower seed (10g/100g and 30g/100g of flour substitution) and hull-less barley flour (30g/100g and 50g/100g levels) had increased in spread ratio as higher supplementation was used.

The hardness of biscuit incorporated with CSP has increased gradually with increased level of CSP. This could be explained by the higher protein composition [35]. CSP may create stronger bonds between the particles in biscuit after baking that could be influenced by protein and water soluble content [35]. The results were in line with the biscuits reported by other studies. As higher flour was substituted with barley flour, the cookies became significantly harder (18.1kg to 20.2kg) than control (11.8kg) [32]. Although the spread ratio among wheat, rice, oat and barley brans were varied but the incorporation of 10 % to 40 % of brans in biscuit had increased their hardness property from 1.38kg to 2.11kg, 1.35kg to 3.83kg, 1.38kg to 1.49kg and 1.58kg to 2.65kg respectively [33]. An increase in hardness of biscuits could be attributable to dilution of gluten and less water involved in hydration of gluten. During dough development, the gluten may become extensive and strong which allow the dough to rise. However, in a harder texture cookie, its dough does not prevent escape of the gas during baking and provide dense texture for the baked product.

Polyphenols and antioxidant activity:

It was observed that the polyphenol content and scavenging capacity of the CSP-based biscuits were significantly increased ($p < 0.05$) as the level of CSP was increased (Table 3). Polyphenol content had significantly increased from 0.42 mg GAE/g in control biscuit to 1.66 mg GAE/g in biscuit with 5% CSP. The content of polyphenols continued to rise to 2.48 mg GAE/g and 4.18 mg GAE/g in biscuit with 10% and 15% CSP respectively. The results also showed that the CSP-based biscuit exhibited comparatively higher DPPH scavenging capacity (24.25% to 62.73%) than the control biscuit (2.51%). In FRAP analysis, the antioxidative capacity of CSP-based biscuit ranged from 16.94 to 342 mg TE/100 g biscuit while there was no FRAP capacity recorded in control biscuit.

Biscuit incorporated with CSP showed significantly higher composition of gallic acid ranging from 21 $\mu\text{g/g}$ to 107 $\mu\text{g/g}$ than the control biscuit (4 $\mu\text{g/g}$) (Table 3). The CSP-based biscuit showed significantly higher concentration of p-coumaric and ferulic acid those were 0.46 to 0.62 $\mu\text{g/g}$ and 2.65 to 6.81 $\mu\text{g/g}$ respectively compared to control. The p-coumaric and ferulic acid of control biscuit were 0.34 $\mu\text{g/g}$ and 0.44 $\mu\text{g/g}$ respectively. However, both catechin and epicatechin compounds were not present in control and CSP-based biscuit.

Table 3: Polyphenol content, DPPH scavenging and FRAP capacity of biscuits.

Parameters	CSP level (%)			
	Control	5%	10%	15%
TPC (mg GAE/g)	0.42 \pm 0.06 ^d	1.66 \pm 0.09 ^c	2.48 \pm 0.15 ^b	4.18 \pm 0.15 ^a
DPPH scavenging activity (%)	2.51 \pm 0.45 ^d	24.45 \pm 2.88 ^c	43.20 \pm 3.15 ^b	62.73 \pm 3.00 ^a
FRAP	Nd	16.94 \pm 1.9 ^c	141 \pm 21 ^b	342 \pm 63 ^a
Gallic acid ($\mu\text{g/g}$)	4.0 \pm 0.1 ^a	21 \pm 2 ^c	61 \pm 8 ^b	107 \pm 5 ^a
P-coumaric ($\mu\text{g/g}$)	0.34 \pm 0.33 ^c	0.46 \pm 0.02 ^b	0.51 \pm 0.03 ^b	0.62 \pm 0.03 ^a
Ferulic acid ($\mu\text{g/g}$)	0.44 \pm 0.01 ^d	2.65 \pm 0.02 ^c	3.00 \pm 0.2 ^b	6.81 \pm 0.03 ^a
Catechin ($\mu\text{g/g}$)	nd	Nd	nd	nd
Epicatechin ($\mu\text{g/g}$)	nd	nd	nd	nd

^{a-d}Superscript with different letter within the same row was significantly different at $p < 0.05$

^a mg TE/100 g biscuit

The incorporation of CSP in biscuit has increased polyphenols content and free radical scavenging capacity of biscuit. the polyphenol content of CSP biscuit found in this study was comparatively higher than the biscuit incorporated with 10% to 30% white grape pomace (2.11 to 4.45 mg GAE/g biscuit) [36]. The polyphenol content of control biscuit was comparatively lower at 0.42 mg GAE/g than CSP-based biscuit at 1.66 – 4.18 mg GAE/g. The DPPH scavenging capacity was ranged from 3.39 to 7.55 mmol TEAC in the white grape pomace biscuit while its control only had scavenging capacity of 1.27 mmol TEAC.

The polyphenol content has increased in accordance with the scavenging capacity. The incorporation of plant sources in bakery products and their roles in radical scavenging activity were also reported by other researchers. Biscuit enriched with 5% to 20% of mango peel powder had higher polyphenols (1800 to 4500 μg GAE/g biscuit) compared to its control biscuit (540 μg GAE/g biscuit) [6]. Moreover, the mango peel biscuit also had stronger DPPH scavenging capacity (IC_{50} = 4.3 to 166 mg) than that of control biscuit (250 mg). The difference in the capacity of radical scavenging capacity of these biscuits therefore was reported to be attributable to the polyphenols and carotenoids [6].

In the present study, the CSP-based biscuit contained gallic acid ranging from 21 to 107 $\mu\text{g/g}$, p-coumaric ranging from 0.46 to 0.62 $\mu\text{g/g}$ and ferulic acid (2.65 to 6.81 $\mu\text{g/g}$). Therefore, it is suggested that higher DPPH scavenging and FRAP capacity shown in CSP-based biscuits were contributed by these polyphenol compounds. The antioxidant capacity of baked foods still remained detected due to the existence of thermal-induced degradative products of original phenolics [37]. Catechins compounds however were not detected in all biscuits incorporated CSP. This may possibly due to dilution effect when incorporated in biscuit formulation thus making them undetected. It was reported that strong reducing power, scavenging capacity and chelating ability on ferrous ions shown by cake added with green tea catechin powder (10% to 30% of flour substitution) was related to incorporation of phenolic compounds namely the catechins [38].

Conclusion:

The incorporation of cornsilk in biscuit increases the amount of protein, dietary fiber and ash content compared to biscuit prepared from wheat flour. Considerable higher polyphenol content (60.4% to 86.8%) and antioxidative improvement has attained by incorporation of cornsilk in biscuits. The CSP-based biscuits have stronger free radical scavenging capacity (24.45% to 62.73%) and ferric reducing capacity (16.94 to 342 μmol TE/g) with higher gallic acid and ferulic acid compound compared to all-wheat based biscuit. Biscuit of high quality can be prepared by incorporating up to 15% of cornsilk with wheat flour substitution.

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